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HORMONE REPLACEMENT THERAPY

10 In the post-menopausal years, women often receive hormone replacement
therapy. This can involve administration of estrogen and progestin or estrogen only.
For women with a uterus, estrogen-only therapy has been associated with increased
5 risk of endometrial cancer. To offset the involved estrogen-induced proliferation of
the endometrium, progestin has been administered. However, the combination of
estrogen and progestin has the undesirable side effect of uterine bleeding which can
reduce the rate of patient compliance.

SUMMARY OF THE INVENTION

10 This invention relates to a method of hormone replacement therapy (HRT)
which comprises administering to a woman in need of such therapy, estrogen in an
hormone replacement therapy effective amount and antiprogestin in an amount which
is effective both to inhibit estrogen-induced endometrial proliferation and to effect
a state of substantial amenorrhea, in the absence of progestin administration.

15 Suitable estrogens, amounts and regimens are in accordance with conventional
considerations for HRT therapy. Examples of estrogens which can be employed in
this invention are ethinyl estradiol and estradiol and their esters, e.g., acetate,
valerate, benzoate and undecylate, mestranol and conjugated estrogens.
Administration can be by any route, e.g., orally or transdermally. For example, the
20 amount of conjugated equine estrogen administered is analogous to that practiced in
conventional estrogen replacement therapy and is generally in the range of about 0.3
to 1.2 mg, preferably about 0.625 to 0.9 mg daily. The determination of an
effective dose is routine, taking into account the usual physical parameters, such as

weight, age and the like, and is best determined by the attending clinician. The administration can be periodic or continuous. The latter, e.g., daily administration, is preferred because individuals are more likely to follow the treatment regimen and not to forget or overlook a periodic administration schedule.

5 Suitable antiprogestins include progesterone receptor antagonists or inhibitors of the biological activity of progesterone, and the like. Examples of antiprogestins which can be employed in this invention are RU 486 ("Mifepristone," Roussel Uclaf, Paris); and "Onapristone" (Schering AG, Berlin; U. S. Patent No. 4,780,461) and the steroids described in the examples and the following patents and patent
10 applications: U. S. Patent No. 4,609,651, especially the compound lilopristone (11 β -(4-dimethylamino-phenyl)-17 β -hydroxy-17 α -(3-hydroxy-prop-1-(Z)-enyl)-4,9(10) estradien-3-one); U. S. application Serial No. 06/827,050, especially the compounds 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1-propinyl)-4,9-estradien-3-one and 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(3-hydroxy-1(2)-propenyl)-4,9-estradien-3-one;
15 U. S. application Serial No. 07/283,632; U. S. application Serial No. 07/541,806, corresponding to published European patent application EP-A 04042831; and other antigestagens, e.g., U. S. Patent 4,891,368. The antiprogesterin can be administered by way of any art recognized means as practiced in the pharmaceutical arts. For example, a suitable antiprogesterin may be formulated so that it can be administered
20 orally, via a skin patch for transdermal absorption, contained within an inert matrix which is implanted within the body and in the depot state or intravaginally in a matrix that slowly releases the antiprogesterin.³ (Such an implant is taught in U. S. Pat. Nos. 4,957,119 and 5,088,505 and the like.)

25 Suitable pharmaceutical formulations are highly conventional and disclosed, e.g., in the various references mentioned herein and elsewhere. The estrogen and antiprogesterin components can be administered separately or in the same dosage form, e.g., tablet.

30 The pharmaceutical formulations may be provided in kit form containing a plurality of, generally at least about 20, and preferably in multiples of 7 such as 28, tablets, intended for ingestion on successive days. Where administration of the antiprogesterin is intended to be periodic, a plurality, generally at least three, of non-

adjacent tablets contain the antiprogestin while the remaining tablets are placebo. Where convenient, the kit may provide the estrogen and antiprogestin can be in the same tablet.

5 The antiprogestin can be administered essentially continuously or periodically, e.g., intermittently. The two types of regimens are equivalent. When the antiprogestin is administered intermittently, higher doses, of course, will be administered in comparison to a continuous antiprogestin regimen. Suitable intermittent dosages include 50-500 mg over 1-3 days, e.g., about 1-10 mg/kg. Administration periods can be weekly, biweekly, every 20 days, monthly, etc. For
10 more continuous regimens, e.g., weekly or more frequently, e.g., daily, every other day, etc., amounts are generally about 0.005 to 1 mg/kg and preferably about 0.05 to 0.5 mg/kg, daily in the case of RU 486. Other milligram amounts may be appropriate in the case of different antiprogestins. Regimens of estrogen and antiprogestin, other than daily and/or in which the dosage amount of the estrogen
15 and/or antiprogestin is periodically varied are also within the scope of the invention. Daily administration is preferred for ease of compliance. For RU 486, a suitable human oral dose would be on the order of about 0.5 to 10 mg per dose, preferably about 1 to 5 mg per dose daily. This amount can be lowered or increased based on the particular regimen utilized and the usual characteristics involved., e.g., the
20 nature of the individual.

 In all cases, the amount of antiprogestin administered will be an amount which inhibits estrogenic endometrial proliferation and enables achievement of amenorrhea. The amenorrhea state established by this invention is substantially, but not necessarily totally, complete. Thus, it will be appreciated that a minor amount
25 of periodic bleeding or spotting on a monthly or yearly basis can occur.

 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way
30 whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

5 The entire disclosure of all applications, patents and publications, cited above and below.

EXAMPLES

Example 1

30 ovariecomized cynomolgus monkeys were studied intensively over 12 weeks. Primates in Group I (N=5 each) received only the vehicles used for hormone delivery in Groups II-VI. Monkeys in Group II received estradiol at physiologic levels throughout (days 1 to 81) via a sc silastic implant, as well as two separate courses of progesterone at physiologic levels via a sc silastic implant (days 28 to 35 and 67 to 74). Groups III-VI received the same sequential regimen of estradiol and progesterone as Group II, but in addition were given an antiprogesterin im on alternate days 39 to 67: Group III, mifepristone (RU 486) 2.0 mg/kg; Group 15 IV, mifepristone 20.0 mg/kg; Group V, onapristone (ZK 299) 2.0 mg/kg; and Group VI, onapristone 20.0 mg/kg. The two menstrual challenge tests (A and B) were defined as the six days immediately after withdrawal of progesterone treatment (A on days 36 to 41 and B on days 75 to 80).

20 The protocol thus provided two periods of estrogen-only HRT treatment, one without antiprogesterin followed by another with antiprogesterin. Each of these two periods was followed by short-term progesterone treatment which provided a measurement of the effects on bleeding of the antiprogesterin during the estrogen HRT treatment.

25 Additional data were collected by measurements of estradiol, progesterone and cortisol, as well as each antiprogesterin in serum drawn on alternate days (1 to 81). Also, endometrial response was examined histologically in biopsy specimens collected on days 53 and 70, both to measure thickness of endometrial tissue (i.e., anti-proliferative response) and to assess the endometrial tissue qualitatively 30 (atrophic, proliferative, or secretory, etc.).

Antiprogesterin Compounds

Onapristone (ZK 299) (1β -(4-dimethylaminophenyl)-17-hydroxy-17 β -(3-hydroxypropyl)-13-methyl-4,9-gonadien-3-one) and mifepristone (RU 486) (11β -(4-dimethylaminophenyl)-17 β -hydroxy-1-propinyl-4,9 estradien-3-one) were dissolved
5 in benzyl benzoate, then mixed with castor oil (2.3, vol:vol). Both antiprogesterins were synthesized at Schering AG (Berlin, Germany).

Primate Model

Thirty normally cycling adult female cynomolgus monkeys were ovariectomized at least 45 days prior to the initiation of this study.

10 The monkeys were randomly distributed into six treatment group preparations (n=5 each). Group I (control) was treated with the vehicle only. One empty silastic implant was placed sc surgically from study day 1 to 81; this controlled for estradiol treatment. Another empty silastic implant was inserted sc from days 28 to 35 and from days 67 to 74 and controlled for progesterone treatment. One ml of
15 the antiprogesterin vehicle was administered im on alternate days (39 to 67). Group II received continuous estradiol progesterone at two intervals but no antiprogesterins. This experimental design allowed two menstrual challenges (A and B). As described below (Groups III-VI), menstrual challenge A was without antiprogesterin exposure; whereas B was with antiprogesterin treatment. Group III monkeys received the
20 sequential estradiol and progesterone regimens as in Group II but also RU 486 at 2.0 mg/kg on alternate days for four weeks (days 39 to 67). For Group IV, the same treatment occurred, except that the RU 486 dose was elevated by ten-fold (20.0 mg/kg). Similarly, primates in Groups V and VI were injected with onapristone at 2.0 and 20.0 mg/kg, respectively.

25 Endometrial Biopsies and Tissue Characterization

In all treatment groups, observations for menstrual bleeding were made daily by visual inspection of the vaginal labia. Femoral blood collections (4.0 ml) were taken under ketamine-induced anesthesia (10 mg/kg) on alternate days and endometrial biopsies (by hysterotomy) were obtained on days 53 and 70.
30 Endometrial biopsies were placed in formalin by staining and processed for histologic evaluation by a clinical pathologist. Thickness of the endometrial tissues

was measured with an ocular micrometer to determine the depth of the endometrium at its point of maximal thickness from the myometrial junction to the epithelial layer at the luminal surface. Endometrial status was evaluated qualitatively and classified according to the following score: atrophic:0; early proliferative: 1; late proliferative:2; interval:3; early secretory:4; and late secretory:5.

Steroid Determination

Blood samples were permitted to clot; serum was harvested and stored at 20°C. RIA's were performed for mifepristone, estradiol, progesterone and cortisol. Onapristone was quantified by HPLC with the detection limit of 4 ng/ml.

Statistical Analysis

Results were expressed as the mean + SD. Statistical comparisons were made. The results are shown in Tables 1 and 2.

The results illustrate that the incidence of withdrawal bleeding during the six days after removal of progesterone challenge in menstrual period A (estrogen(E)-only, no antiprogestin(AP)) was 31.3% (47 of 150 days, Groups II-VI combined), while zero among Group I primates (vehicle controls). For menstrual period B, again the Group I control Monkeys had no bleeding episodes. Also sustained was a 30.0% (9 of 30 days) bleeding incidence among estradiol plus progesterone only treated control monkeys (Group II). In contrast, E-only plus antiprogestin treatment (Groups III-VI) manifested only a 2.5% (3 of 120 days) combined incidence of withdrawal bleeding. These three incidents of induced menses all occurred in association with onapristone at the lower dose of 2.0 mg/kg. Using mifepristone at either 2.0 or 20.0 mg/kg, or onapristone at 20.0 mg/kg, uniformly led to complete amenorrhea during menstrual challenge B. These differences in bleeding rates for E-only HRT without versus with antiprogestin exposure are highly significant ($P < 0.01$, combined groups).

Endometrial growth (thickness) at day 53 was approximately 2.5 mm in all treatment groups (II to VI), versus vehicle controls at about 0.5 mm. However, on day 70, among the therapeutic regimens of Groups III-VI, thicknesses of endometrial

Table 1, Incidence of withdrawal menses^a after sequential estradiol^b and progesterone^c therapy without versus with antiprogesterin treatment^d

Experimental Design	Menstrual Challenge A (Days)		Menstrual Challenge B (Days)	
	Antiprogesterins: None Progesterone : 28 - 35 Withdrawal Interval: 36 - 41		Antiprogesterins: 39 - 67 Progesterone: 67 - 74 Withdrawal Interval: 75 - 80	
Treatment Groups (N=5)	Incidence of Bleeding		Incidence of Bleeding	
	Days	%	Days	%
I. Vehicle Control	0/30	0	0/30	0
II. Estradiol + Progesterone only	4/30		9/30	30
III. Estradiol + Progesterone + MIF @ 2 mg/kg	13/30		0/30	
IV. Estradiol + Progesterone + MIF @ 20 mg/kg	12/30	47/150	0/30	3/120
V. Estradiol + Progesterone + ONA @ 2 mg/kg	9/30	31.3	3/30	2.5
VI. Estradiol + Progesterone + ONA @ 20 mg/kg	9/30		0/30	

- a) Menses is defined here to be two or more consecutive days of blood observed on the vaginal labia within six days after withdrawal of progesterone treatment.
- b) Estradiol was administered via sc silastic implant throughout the study (Groups II - VI).
- c) Progesterone was administered via sc silastic implant in two sequential regimens of seven days each.
- d) Anti-progesterins (Mifepristone and ONA-pristone) were injected im on alternate days 39 - 67 (Groups III - VI).

Table 2. Histologic evaluation of primate endometrial tissues on days 53 and 70						
	Group I	Group II	Group III	Group IV	Group V	Group VI
<u>Day 53</u>						
Thickness (mm)	0.4 ± 0.1	2.6 ± 0.1*	2.5 ± 0.2*	2.2 ± 0.2*	2.6 ± 0.2*	2.2 ± 0.1*
Endometrial Score	0	4.0	3.6	3.8	3.8	3.6
<u>Day 70</u>						
Thickness (mm)	0.5 ± 0.1	2.7 ± 0.1	1.8 ± 0.2**	1.3 ± 0.1**	1.6 ± 0.1**	1.7 ± 0.1**
Endometrial Score	0	4.3	2.4	2.8	4.0	3.0

Score:

- 0 - atrophic
 - 1 - proliferative
 - 2 - late proliferative
 - 3 - interval
 - 4 - early secretory
 - 5 - secretory
- * Statistically significant differences ($p < 0.05$) from controls (Group I)
- ** Statistically significant differences ($p < 0.05$) from controls (Group I) and sequential estradiol/progesterone only (Group II)

tissues were significantly ($P < 0.05$) diminished after exposure to the anti-proliferative influences of RU 486 and onapristone (approximately 1.5 mm) compared to the absence of antiprogesterin in Group II (2.7 mm). This effect was most profound in the high dose, RU 486 group (IV, 20.0 mg/kg). Upon histological evaluation, estradiol and progesterone alone had induced development of lush secretory endometrium present at both days 53 and 70 (Group II). Similarly, the low dose of onapristone (Group V, 2.0 mg/kg) manifested well-developed secretory glands, even though the tissue thickness was reduced (1.6 mm); this is the same group of monkeys in which only days (three) of withdrawal bleeding were observed after antiprogesterin administration (menstrual challenge B). The other primates (Groups III, IV and VI) displayed differential endometrial states, ranging from late proliferative to early secretory characteristics, in association with tissue compaction (reduced endometrial thickness), as well as maintaining amenorrhea uniformly during menstrual challenge B.

These data show that E-only + continuous AP treatment was effective to achieve no breakthrough bleeding throughout the entire study, i.e., it induced a state of sustained amenorrhea and also displayed antiproliferative effects (weak) on the endometrium.

EXAMPLE 2

The purpose of this study was to evaluate the antiproliferative action of antiprogesterins on the endometrium in estrogen-replaced, ovariectomized cynomolgus monkeys.

Previously ovariectomized cynomolgus monkeys had implanted subcutaneously a 3 cm estradiol containing silastic capsule on day 1 of the study. This capsule was removed on day 30. The vehicle or antiprogesterin was administered intramuscularly on a daily basis from day 1 to 30. Vaginal swabs were performed daily from the first day of treatment. Femoral blood samples (3.5cc) were collected under ketamine-induced anesthesia (10 mg/kg, im) on day 1, 8, 15, 22 and 30 for analyses of estradiol, cortisol and antiprogesterin. An endometrial biopsy was obtained by hysterotomy on day 30 under ketamine (20 mg/kg)/xylazine (1 mg/kg) anesthesia.

Nubain (1 mg/kg) was administered post-operatively for analgesia every twelve hours as needed. The endometrial biopsy was processed for histology; the specimens were classified by developmental stage, endometrial thickness, PCNA, K167, ER and PR.

5 The treatment groups, with three monkeys per group, were as follows:

1. Myrj - vehicle control, im
2. ZK136798 1 mg/kg, im
3. ZK136798 3 mg/kg, im
- 10 4. ZK137316 1 mg/kg, im
5. ZK137316 3 mg/kg, im
6. Onapristone 1 mg/kg, im
7. Onapristone 3 mg/kg, im
8. RU 486 1 mg/kg, im
9. RU 486 3 mg/kg, im

15 (ZK numbers are in-house designations of Schering, AG, Berlin, Germany.)

The endometrial specimens from monkeys treated with ZK137316 were primarily classified as "regressed-proliferative." In contrast, the other groups were primarily classified as control - early secretory:

- 20 1. ZK136798 - interval
2. Onapristone - interval
3. RU 486 - proliferative

The specimens classified as "regressed proliferative" had proliferative characteristics but had reduced glandular size and dense stroma. The thickness of the endometrium from antiprogestin treated monkeys was less than that of the vehicle-treated control monkeys.

25 The number of mitotic spindles per ten glands was as follows:

1. control -2
2. Onapristone - 0.7 to 1.0
3. ZK136798 - 0.5 to 1.0
- 30 4. RU 486 - 0.0 to 0.3
5. ZK137316 - 0.0

There were no meaningful differences between groups relative to PCNA labeling. PCNA activity and mitotic activity become disassociated with antiprogestin

treatment. Of the antiprogestins evaluated in this continuous administration mode, ZK137316 appears to be the most effective in inhibiting endometrial proliferation.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.